

What is claimed is:

1. A method for determining amino acid sequence of a peptide, comprising the steps of:

preparing a peptide of interest or fragments thereof
5 obtained by optionally cleaving the peptide of interest;

coupling an amino acid derivative to the N-terminus of the peptide of interest or the fragments thereof, the amino acid derivative having protected an amino group with a protective group and derived from an amino acid with a side chain containing
10 an acidic group; and

subjecting the coupled product to mass spectrometry analysis.

2. The method according to claim 1, wherein the acidic group is selected from the group consisting of carboxyl, sulfo,
15 phosphono, sulfate, and phosphate group.

3. The method according to claim 1, wherein the amino acid is selected from the group consisting of cysteic acid, aspartic acid, glutamic acid, threonine phosphate, serine phosphate, tyrosine sulfate, and tyrosine phosphate.

20 4. The method according to claim 1, wherein the protective group is a functional group other than a basic group.

5. The method according to claim 1, wherein the protective group is selected from the group consisting of biotinyl, acetyl, formyl, and phenylisothiocarbamyl.

25 6. The method according to claim 1, wherein the protective

group is biotinyl.

7. The method according to claim 1, wherein the amino acid derivative is N-biotinylcysteic acid.

8. The method according to claim 1, wherein the cleaving is
5 performed by using an enzyme that can specifically hydrolyze a peptide bond on the C-terminal side of a basic amino acid residue.

9. The method according to claim 1, wherein the peptide or the peptide fragment coupled to the amino acid derivative is
10 ionized and is decayed into decay ions, which are then subjected to mass spectrometry for separation and detection.

10. The method according to claim 9, wherein the peptide or the peptide fragment coupled to the amino acid derivative is ionized by matrix-assisted laser desorption ionization
15 (MALDI).

11. The method according to claim 9, wherein the ions are separated and detected by time-of-flight mass spectrometry (TOFMS).

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